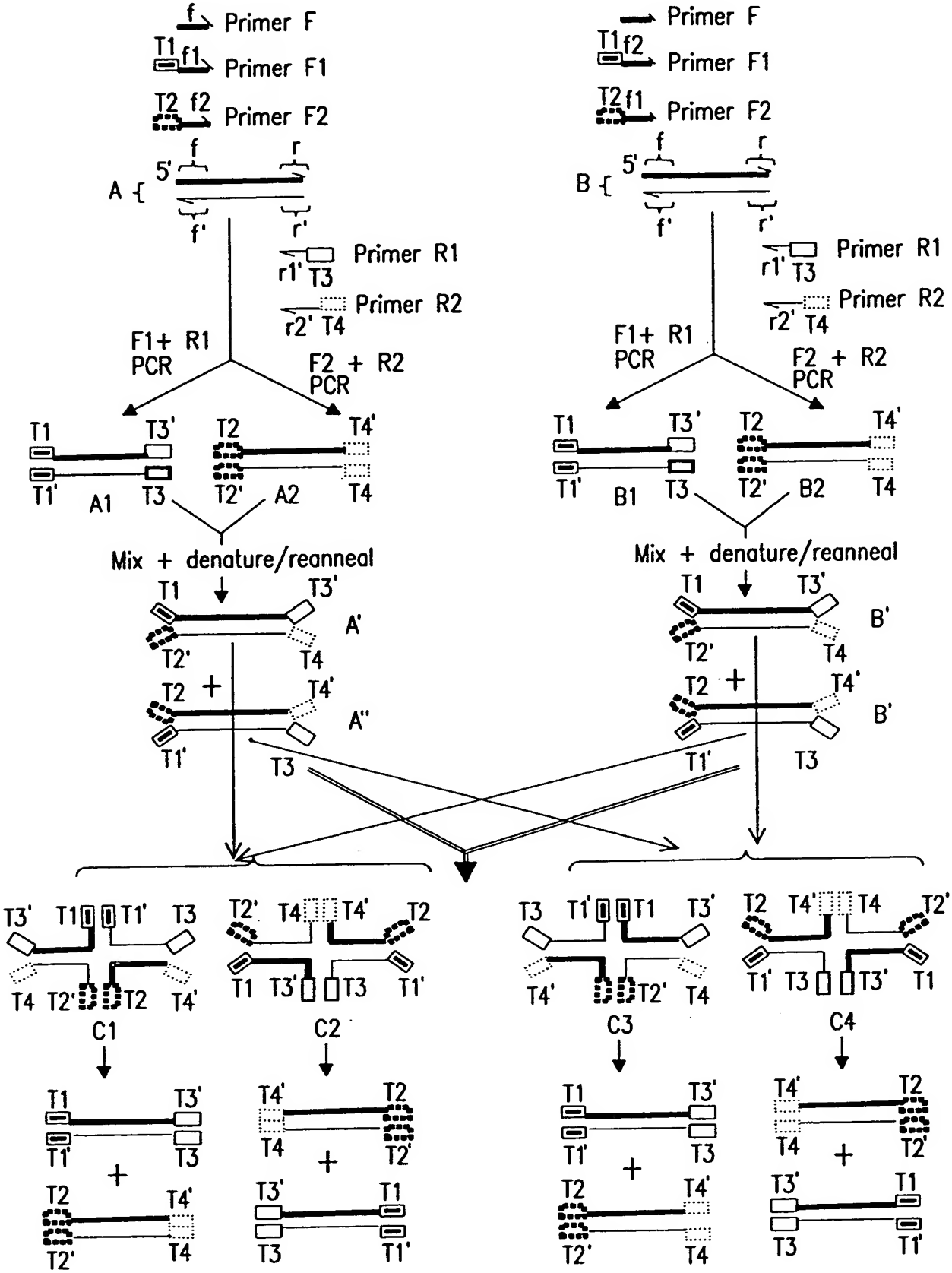

Primer F
Primer F1
Primer F2
Primer R1
Primer R2
A
B
5'
f
r
f'
r'
r1' T3
r2' T4
T1
f1
T2 f2
F1+ R1 PCR
F2 + R2 PCR
T3'
T2'
T4'
T1'
A1
A2
B1
B2
Mix + denature/reanneal
A'
A''
B'
C1
C2
C3
C4

FIG. 1

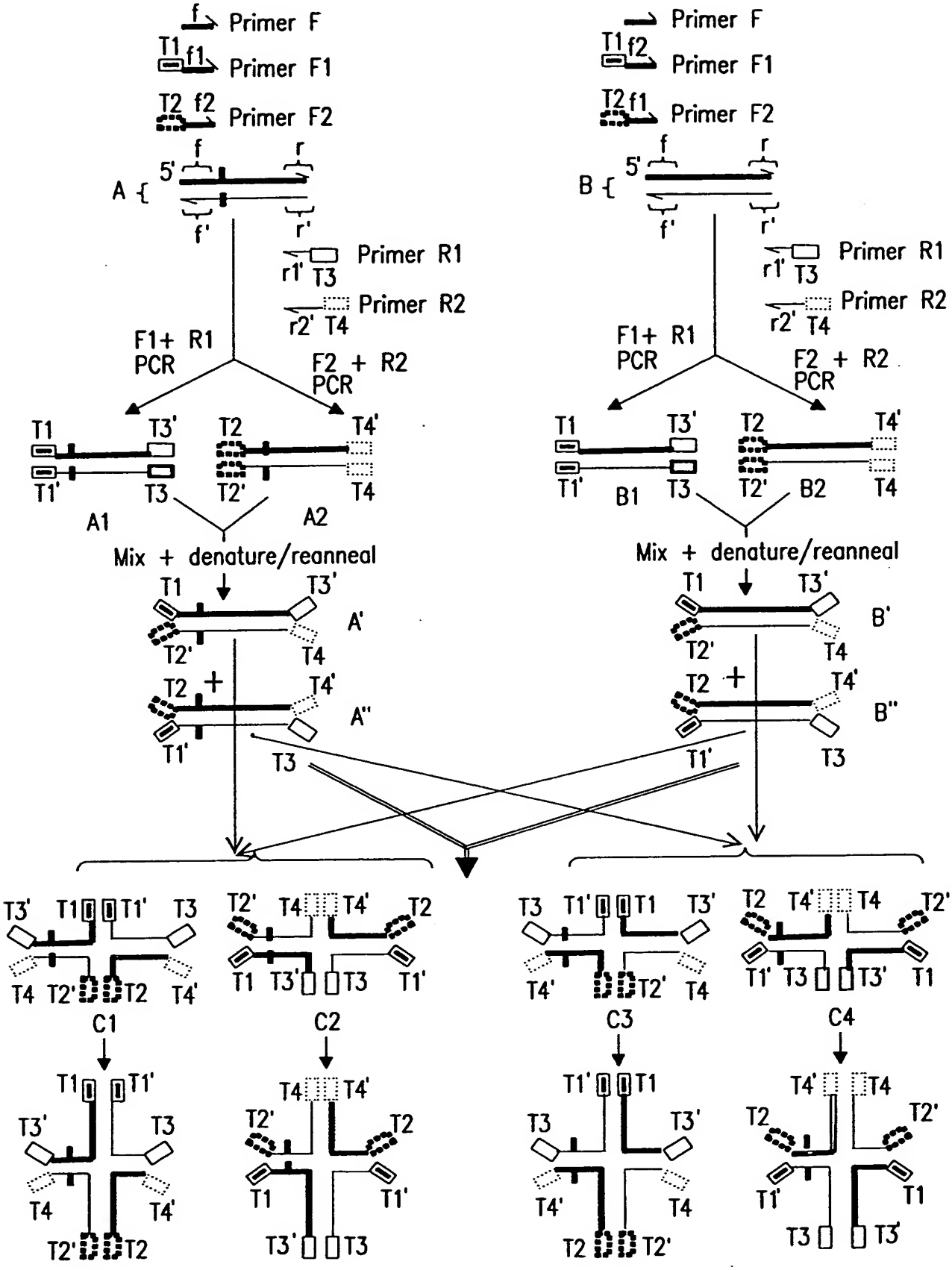

Primer F
Primer F1
Primer F2
Primer R1
Primer R2
F1+ R1 PCR
F2 + R2 PCR
Mix + denature/reanneal
A
B
A1
A2
B1
B2
A'
A''
B'
B''
C1
C2
C3
C4

FIG.2

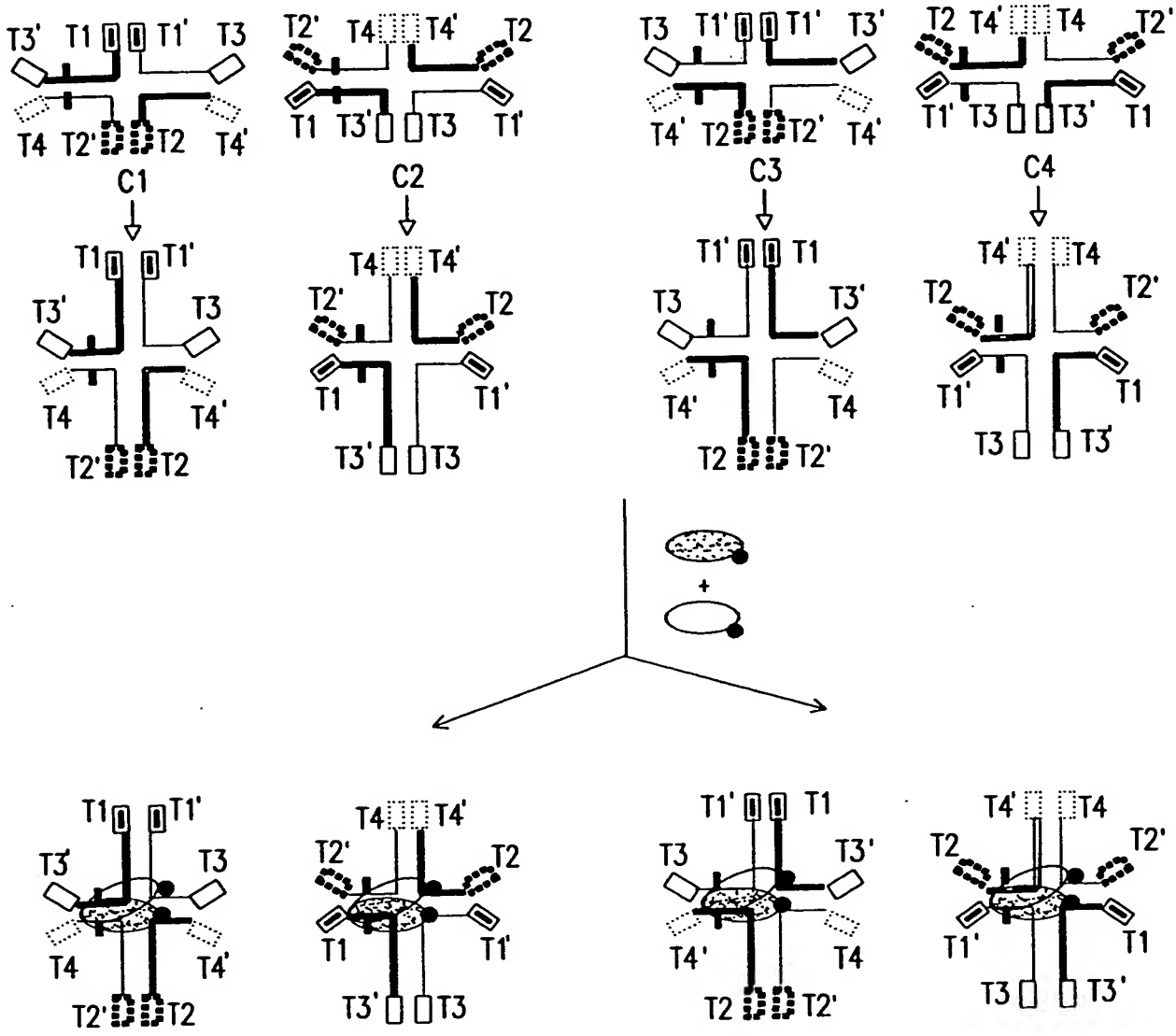

T3' T1 T1' T3
T4 T2' T2 T4'
C1
T1 T1'
T3' T3
T4 T4'
T2' T2
T2' T4 T4' T2
T1 T3' T3 T1'
C2
T4 T4'
T2' T2
T1 T1'
T3' T3
T3 T1' T1' T3'
T4' T2 T2' T4'
C3
T1' T1
T3 T3'
T4' T4
T2 T2'
T2 T4' T4 T2'
T1' T3 T3' T1
C4
T4' T4
T2 T2'
T1' T1
T3 T3'
+
T1 T1'
T3' T3
T4 T4'
T2' T2
T4 T4'
T2' T2
T1 T1'
T3' T3
T1' T1
T3 T3'
T4' T4
T2 T2'
T4' T4
T2 T2'
T1' T1
T3 T3'

FIG.3

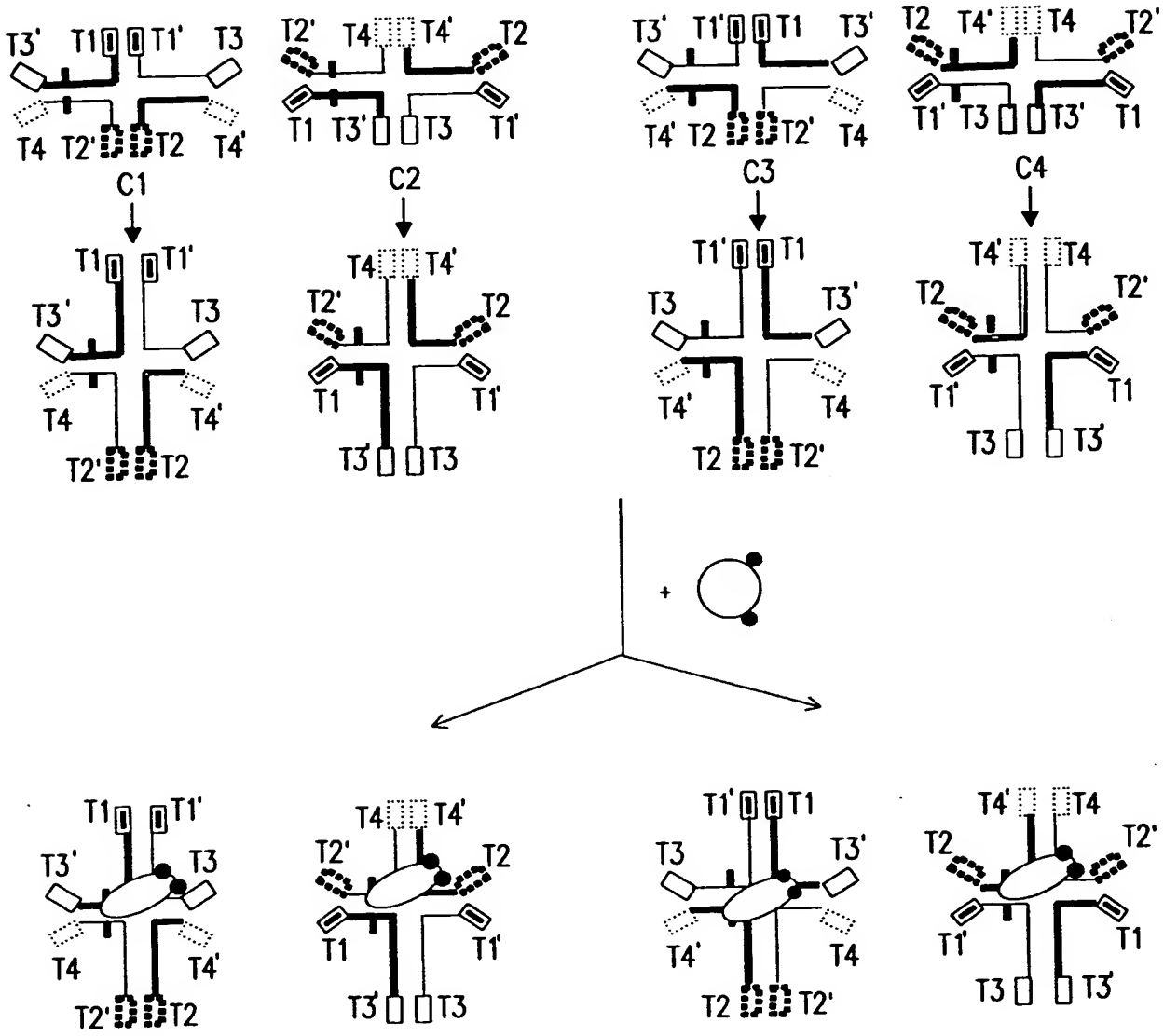

T3' T1 T1' T3
T4 T2' T2 T4'
C1
T1 T1'
T3' T3
T4 T4'
T2' T2
T2' T4 T4' T2
T1 T3' T3 T1'
C2
T4 T4'
T2' T2
T1 T1'
T3' T3
T3' T1' T1 T3'
T4' T2 T2' T4
C3
T1' T1
T3 T3'
T4' T4
T2 T2'
T2 T4' T4 T2'
T1' T3 T3' T1
C4
T4' T4
T2 T2'
T1' T1
T3 T3'
+
T1 T1'
T3' T3
T4 T4'
T2' T2
T4 T4'
T2' T2
T1 T1'
T3' T3
T1' T1
T3 T3'
T4' T4
T2 T2'
T4' T4
T2 T2'
T1' T1
T3 T3'

FIG.4

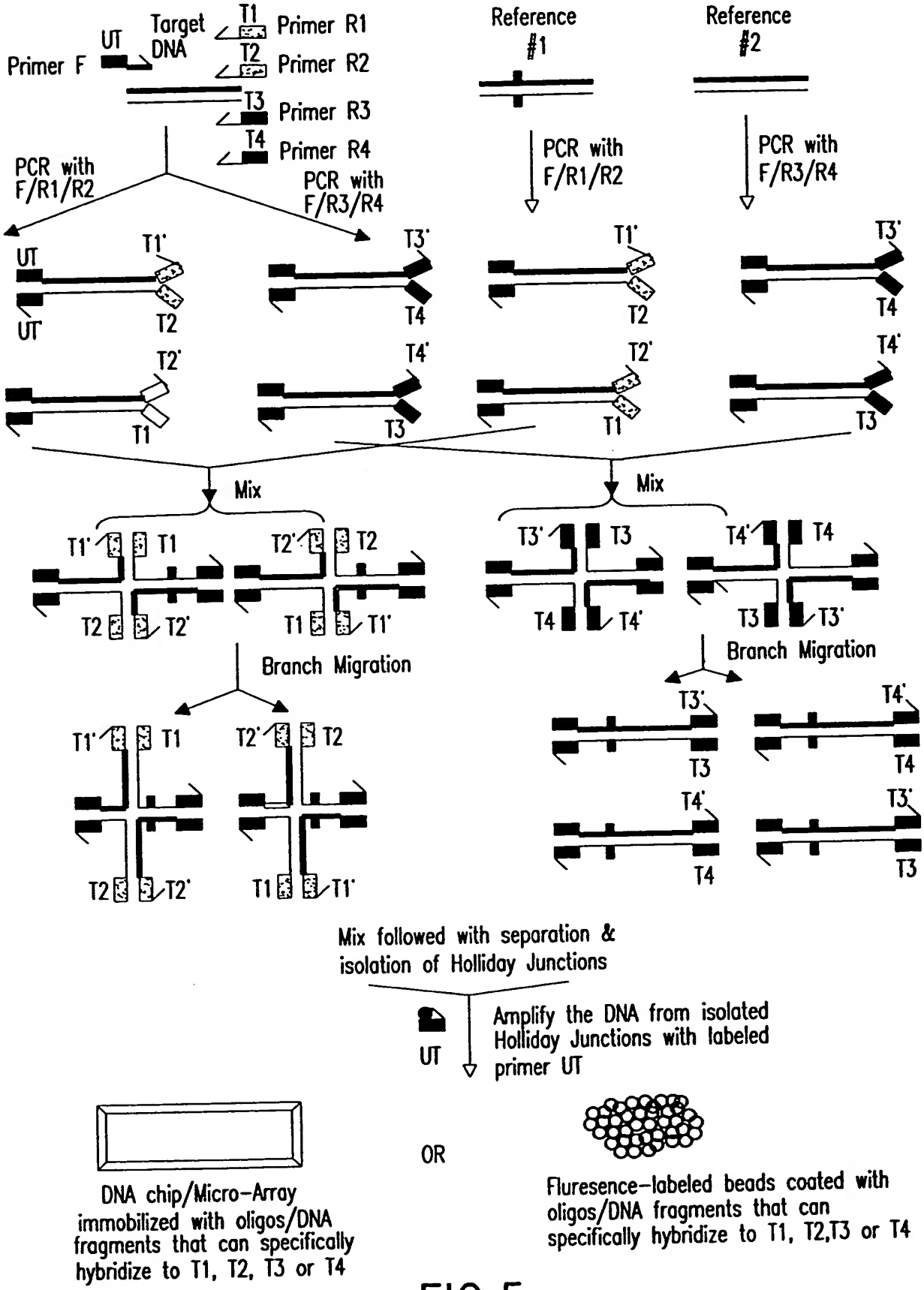

Target DNA
Primer F
UT
T1
Primer R1
T2
Primer R2
T3
Primer R3
T4
Primer R4
Reference #1
Reference #2
PCR with F/R1/R2
PCR with F/R3/R4
PCR with F/R1/R2
PCR with F/R3/R4
Mix
Mix
Branch Migration
Branch Migration
Mix followed with separation & isolation of Holliday Junctions
UT
Amplify the DNA from isolated Holliday Junctions with labeled primer UT
OR
DNA chip/Micro-Array immobilized with oligos/DNA fragments that can specifically hybridize to T1, T2, T3 or T4
Fluresence-labeled beads coated with oligos/DNA fragments that can specifically hybridize to T1, T2,T3 or T4

FIG.5

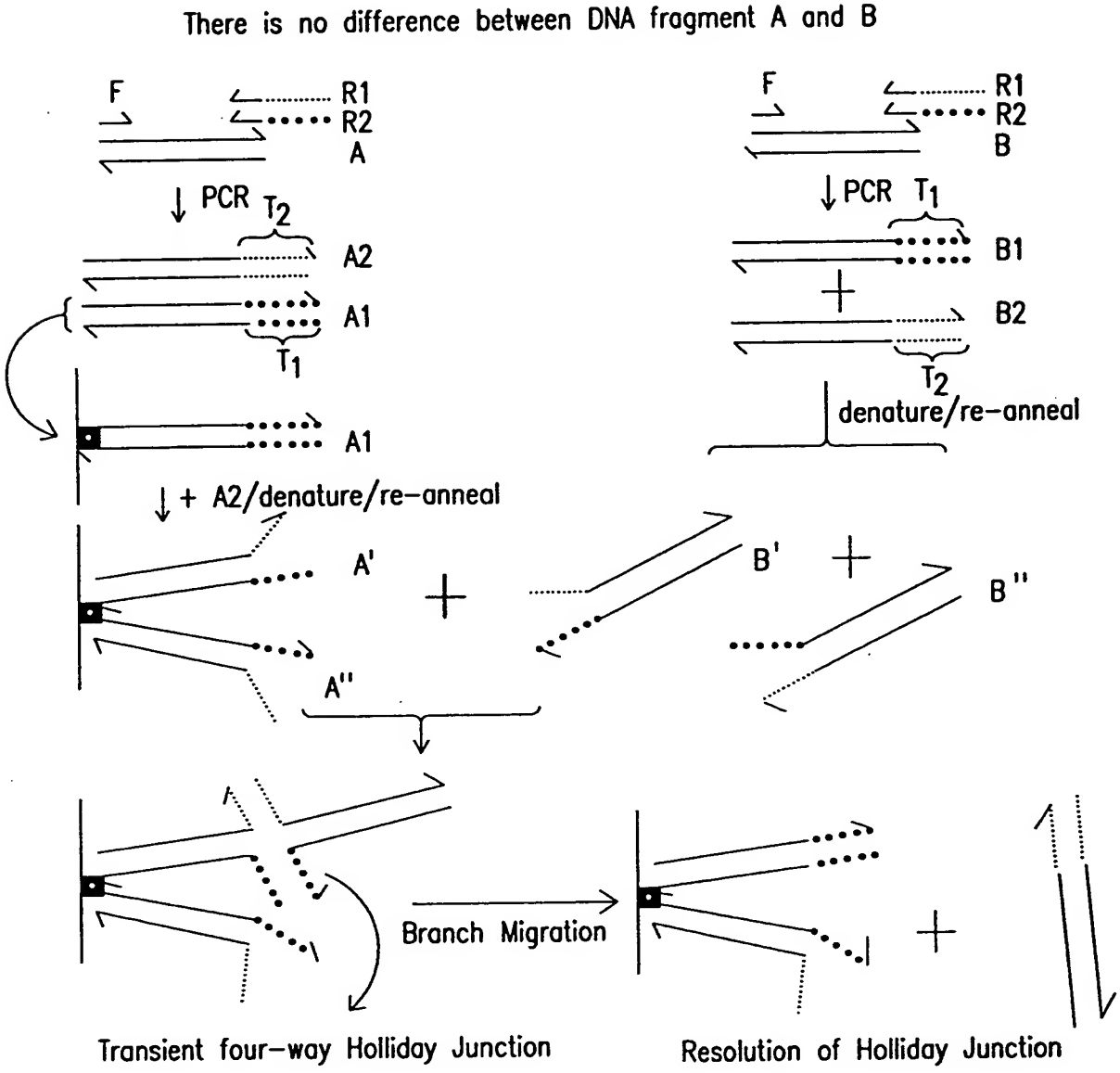

There is no difference between DNA fragment A and B
F
R1
R2
A
PCR T2
A2
A1
T1
A1
+ A2/denature/re-anneal
A'
A''
F
R1
R2
B
PCR T1
B1
B2
T2
denature/re-anneal
B'
B''
Branch Migration
Transient four-way Holliday Junction
Resolution of Holliday Junction

FIG.6A

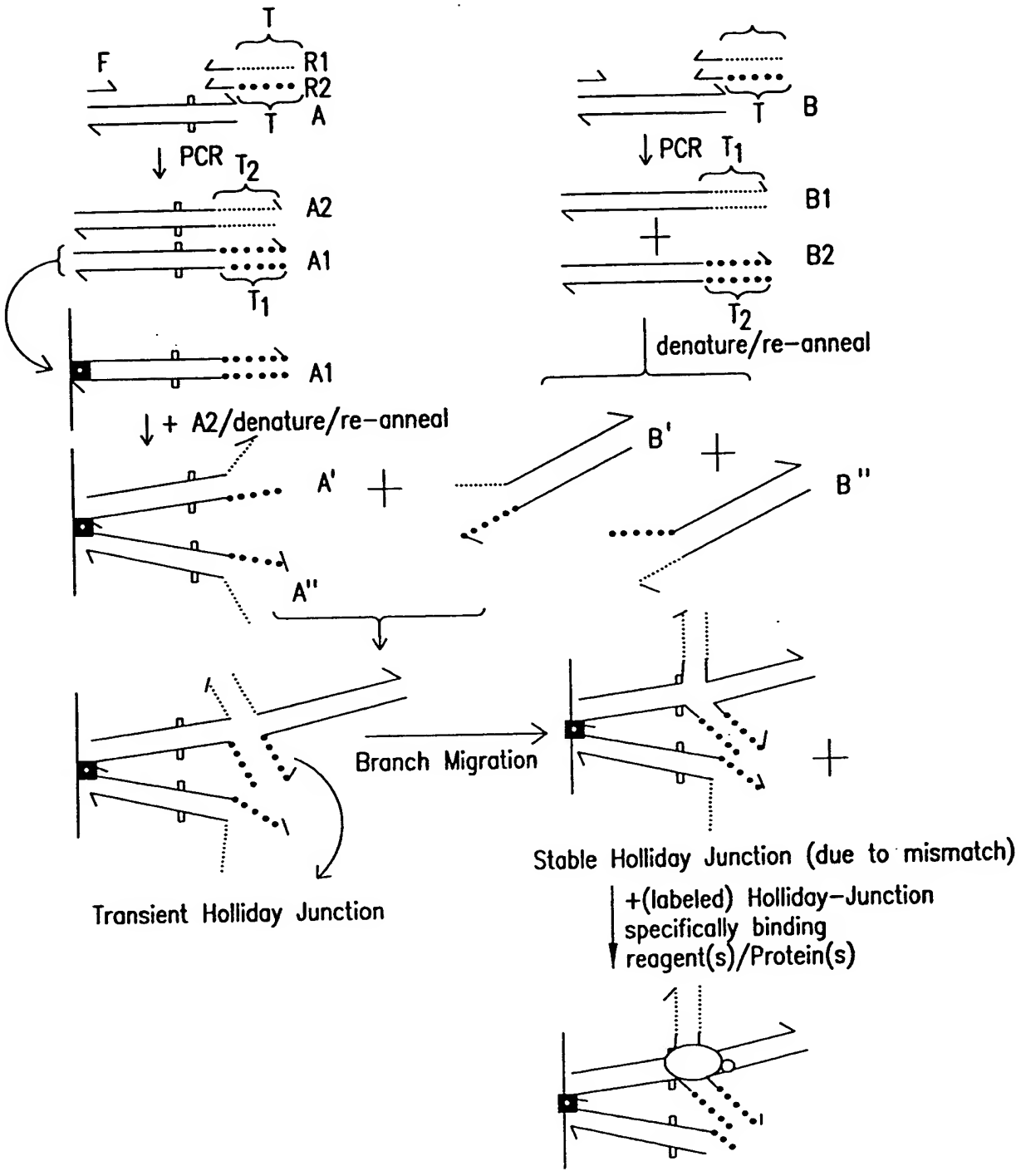
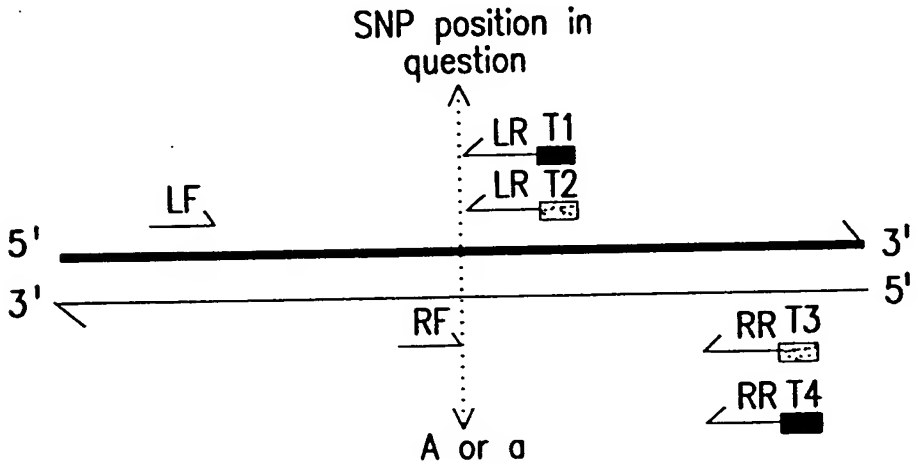

There is a difference between DNA fragment A and B
T
F
R1
R2
T
A
PCR T2
A2
A1
T1
A1
+ A2/denature/re-anneal
A'
A"
T
B
PCR T1
B1
B2
T2
denature/re-anneal
B'
B"
Branch Migration
Transient Holliday Junction
Stable Holliday Junction (due to mismatch)
+(labeled) Holliday-Junction
specifically binding
reagent(s)/Protein(s)

FIG.6B

For each SNP position in question, two versions exist (bi-allelic): either A or a.

Two sets of primers:

- 1st set of primers: LF/LR1/LR2
- 2nd set of primers: RF/RR1/RR2



T1=/=T2 , T3=/=T4, T1/T2 =T3/T4 or T1/T2 =/=T3/T4

FIG.7

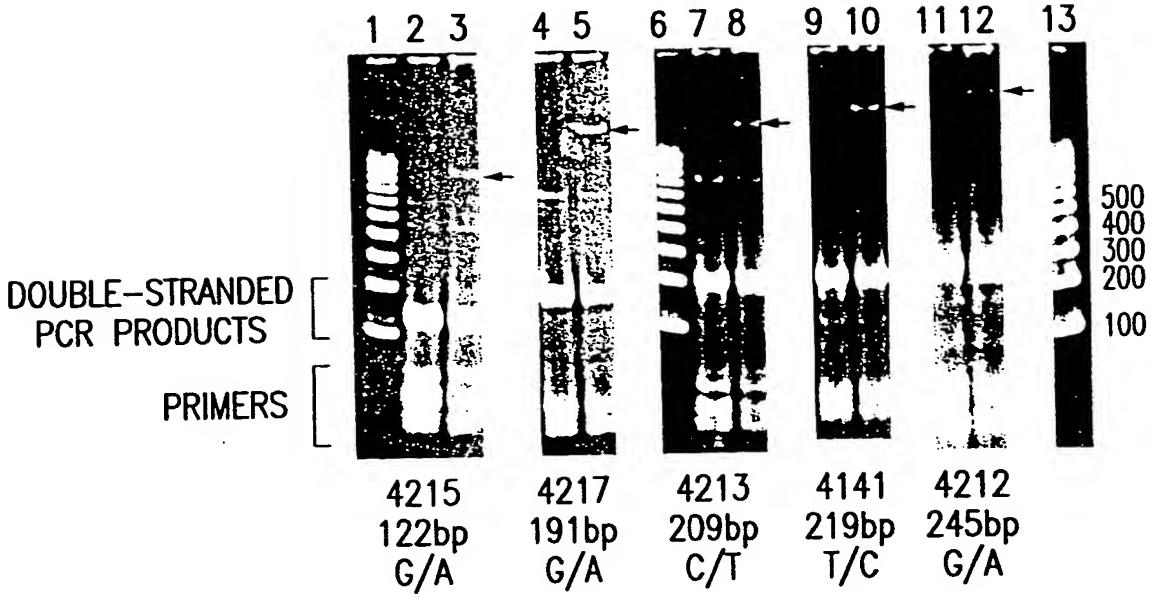

1 2 3
4 5
6 7 8
9 10
11 12
13
DOUBLE-STRANDED PCR PRODUCTS
PRIMERS
500
400
300
200
100
4215 122bp G/A
4217 191bp G/A
4213 209bp C/T
4141 219bp T/C
4212 245bp G/A

FIG.8A

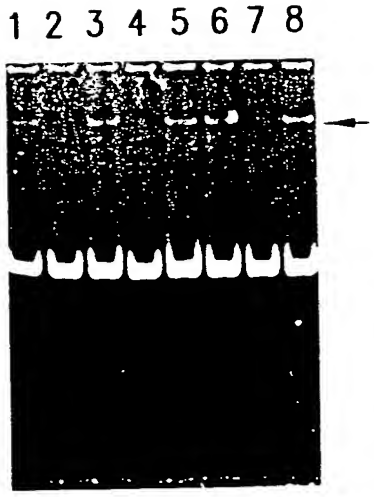

1 2 3 4 5 6 7 8

FIG.8B

FIG.9A

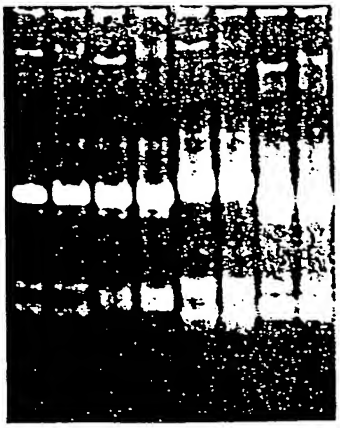


FIG.9B

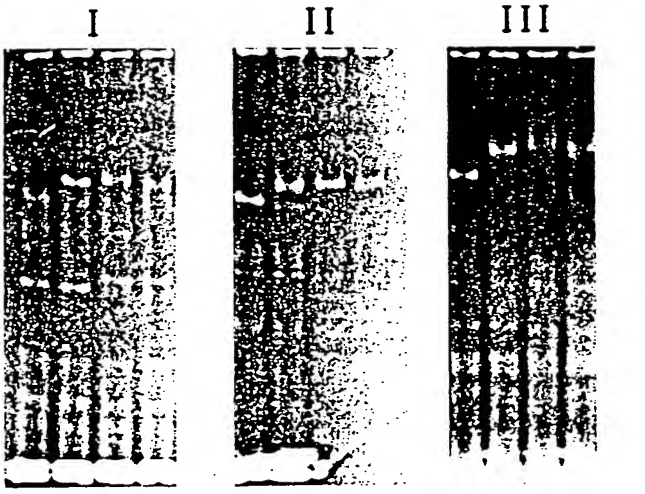


FIG.9C

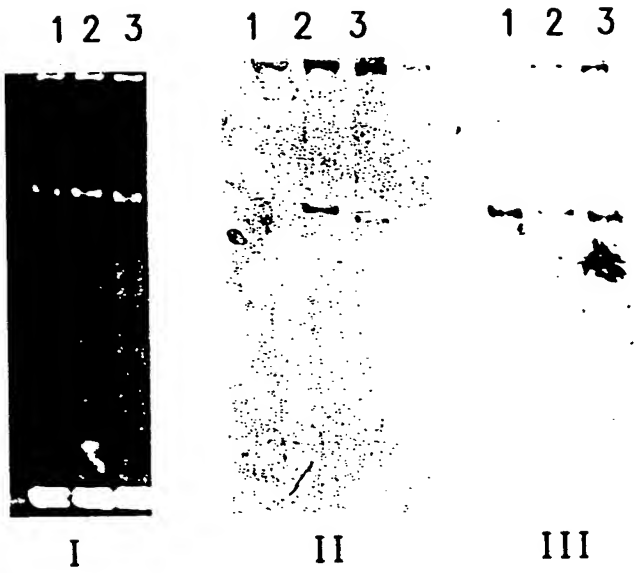


FIG.10